IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Bentwich et al.		Art Unit:	1635
App. No.: 10/536,560		Examiner:	Dana Shin
Filing Date: November 26, 2003		Title:	Bioinformatically Detectable Group of Novel Viral Regulatory Genes and Uses Thereof
§371(c) Filing Date: December 20, 2005			

DECLARATION OF PROF. Alik Honigman UNDER 37 C.F.R. § 1.132

I, Alik Honigman (Ph.D.), hereby declare as follows:

1. I am currently employed as a faculty member at the virology department of the Hebrew University-Hadassah Medical School, Jerusalem, Israel. A true and correct copy of my *Curriculum Vitae* is attached to this declaration as Exhibit A. I am an author of 64 peer-reviewed scientific publications, of which about 33 are related to viral biology. I am familiar with the subject matter disclosed and claimed in U.S. Patent Application No. 10/536,560.

2. I have been informed and understand that the last Office Action issued in this application was dated September 26, 2007 and that the Examiner of this application is of the view and stated in the Office Action that the subject matter claimed in this application is considered unpatentable as obvious over Moss *et al., Current Biology* 12:R138-R140 (2002), in view of Yu *et al., J. of Virology* 73:3638-3648 (1999) and Konings *et al., J. of Virology* 66:632-640 (1992) as well as Grad *et al., Molecular Cell* 11:1253-1263 (2003) and Lagos-Quintana *et al., Science* 294:853-858 (2001) in view of Konings *et al.* I make this declaration to provide facts that I believe are probative of the issues raised by the rejection.

3. The subject matter of this application relates to viral miRNA-related nucleic acids. At the time of invention of this application, miRNAs were known to be processed from hairpin structures. In addition, computational methods such as those described by Moss *et al.* and Grad *et al.* had been used to identify miRNAs by taking into account certain structural features of hairpin precursors. However, these computational methods required sequence conservation to be present with previously known miRNAs. All previously known miRNAs were limited to complex multicellular eukaryote organisms, specifically plants and animals. There are great phylogenic differences existing between complex multicellular eukaryotes and more primitive organisms, which lead to substantial sequence divergences. Due to the computational methods of Moss *et al.* and Grad *et al* requiring sequence conservation, I would have been skeptical that such methods would be able to identify miRNAs in more primitive organisms such as viruses.

4. Regardless of the method used to identify miRNAs, and although logically, from a functional point of view, one could have thought that viruses can benefit from miRNA, I was skeptical that miRNAs existed in viruses. At the time, miRNAs were believed to be extremely rare within the very large genomes of complex eukaryotes. At comparable prevalence within the genome or non-coding sequences, miRNAs would not be present in a virus because a viral genome and viral non-coding sequences are on the order of 10^3 to 10^6 times smaller than the complex multicellular eukaryotes that were known to contain miRNAs.

5. Yu *et al.* found a hairpin secondary structure in the Bovine viral diarrhea virus (BVDV) genome. Despite such a secondary structure, I would have been skeptical that viruses contain miRNAs. Many secondary hairpin structures were known to exist in viruses, however, each of these structures act in *cis*-related regulation (e.g., translation, initiation, IRES, and PKR inhibition) rather than *trans*-related regulation, such as a miRNA. For these reasons, I would have been skeptical that viruses contain miRNAs.

6. In summary, miRNAs known prior to the invention of this application were limited to complex multicellular eukaryotes. There had been no indication that miRNAs existed in more primitive organisms, such as yeast, bacteria, archibacteria or viruses. In fact, in an estimation of the number of miRNAs in the human genome by one of the leaders in the miRNA field, Lim *et al., Science* 299:1540 (2003) states the belief that miRNAs do not exist in more simple organisms such as yeast. Thus, at the filing date of this application, it was believed in the field that miRNAs did not exist in viruses.

7. Pfeffer *et al. Science* 304:734-736 (2004), which was published well after the invention of this application, reports the first public discovery of viral miRNAs. Pfeffer *et al.* was clearly regarded as a significant advance by the scientific community as shown by being published in *Science* and by also meriting a comment in Editor's Choice of *Science.* The editorial comment in *Science* brings attention to the discovery of miRNAs in the "fourth domain of life," indicating that this was recognized as a ground breaking achievement.

8. In accordance with my experience in this field and my knowledge of the state of the art at the time the invention of this application was made, it is clear that the inventors broke with the established teachings in the field to identify miRNAs in simple organisms using computational approaches not requiring sequence conservation. I regard this as a significant advance in the field.

9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-referenced application or any patent issuing thereon.

Dated__02/26/08-- By Prof Alik Honigman

Exhibit A

CV Alik Honigman

CURRICULUM VITAE

Prof. ALIK HONIGMAN

Birth date: 1 January 1942

Marital status: Married, three children

1959-1962: Military service

Academic position: Full Professor

Education

1967, B.Sc., The Hebrew University of Jerusalem, Israel. Faculty of Science. Main Subject: Microbiology and Genetics.

1969, M.Sc., The Hebrew University-Hadassah Medical School, Jerusalem, Israel. Dept. Chemical Microbiology.

Thesis: "Genetic control of the life cycle of Bacteriophage P1"

1970-1975, Ph.D. student, Assistant and Instructor at the, Dept. of Molecular Genetics. The Hebrew University-Hadassah Medical School, Jerusalem, Israel.

Thesis: "Genetics and Biochemical Studies of the early Regulation of Bacteriophage lambda".

1975-1977 McArdle Laboratory for Cancer Research, University Of Wisconsin, Madison, WI - Research Associate in Prof. Szybalski's laboratory.

Positions held

2006 – now Chairman of Planning and Development of the Faculty of medicine of the Hebrew University, Jerusalem

2004- now The Lady Davis Chair in Experimental Medicine and Cancer Research.

2003- now Member in the academic council of the David Yelin College for education in Jerusalem.

2000-now Chairman of the Interdepartmental Equipment Unit of the Faculty of Medicine, the Hebrew University, Jerusalem, Israel.

1996- 2000 Dean Assistant for Public Relations, The HU Faculty of Medicine.

1966- now Chairman of the scientific advisory board of Promega Biotechnology Corporation.

1995- now Dept. of Virology, The faculty of Medicine, The Hebrew University, Jerusalem.

1994-1995 Promega Corp. and the University of Wisconsin, Visiting Professor of Molecular Virology

1992- 1997 Institute of Microbiology - Head of Curriculum Committee

1990-1995 Head of Department of Molecular Genetics, The Hebrew University-Hadassah Medical School, Jerusalem.

1992-1995 Dept. of Molecular Genetics, Hebrew University- Hadassah Medical School, Associate Professor

1983-1992 Dept. of Molecular Genetics, Hebrew University- Hadassah Medical School, Senior Lecturer

1984-1985 NIH, NCI-FCRF, Fredrick, Maryland, Visiting Scientist.

1977-1982 Dept. of Molecular Genetics, Hebrew University- Hadassah Medical School, Lecturer

Books edited

Aids A global Phenomenon (1998) Editors: Morag, A and **Honigman, A,** The Hebrew University of Jerusalem, Akademon, Student Union press.

Publications

1. Olitzki, A., D. Godinger, M. Israeli and **A. Honigman** (1967). Studies on Atypica mycobacteria. Glycols, mono- and polyhydric alcohols as growth Promoting and inhibiting substances. Estratto dal Boll 1st sieroterMilanese 46:5-6

2. Olitzki, A., H. Haas, D. Godinger, M. Israeli and **A. Honigman** (1967).Studies on atypical Mycobacteria in Israel. Path. Microbiol. 30:433-488.

3. Olitzki, A., D. Godinger, M. Israeli and A. Honigman (1967). In vitro Effects of some chemotherapeutic agents on mycobacteria. Applied Microbiology 15:994-1001.

4. Echols, H., L. Green, A.B. Oppenheim and **A. Honigman** (1973). Role of The cro gene in bacteriophage lambda development. J. Mol. Biol. 80:203-216.

5. Oppenheim, A., **A. Honigman** and A.B. Oppenheim (1974). Interference with phage lambda cro gene function by a colicin-tolerant E. coli mutant. Virology 61:1-10.

6. Oppenheim, A.B., **A. Honigman**, A. Oppenheim and W. Stevens (1975).Interaction between bacteriophage lambda and its bacterial host: regulation Of developmental pathways, in: Dynamic Aspects of Host Parasite Relationships, A. Zuckerman, Editor. A. Halsted Press: New York, Toronto, Israel Universities Press, Jerusalem, pp. 40-55.

7. **Honigman, A.,** A. Oppenheim and W. Stevens (1975). A pleiotropic Regulatory mutation in lambda bacteriophage. Mol. Gen. Genet. 138:85-111.

8. **Honigman, A.,** S.L. Hu, R. Chase, and W. Szybalski (1976). 4S oop RNA is a leader sequence for the immunity-establishment transcription in coliphage lambda. Nature 262:112-116.

9. Rosenvold, E. and **A. Honigman** (1977). Mapping of Ava 1 and Xma 1 cleavage sites in bacteriophage lambda DNA including a new technique of DNA digestion in agarose gels. Gene. 2:273-288.

10. Fiandt, M., W. Szybalski, **A. Honigman** and E. Rosenvold (1977). Precise measurement of the b2 deletion in *E. coli* phage lambda. Gene. 3:289-293.

11. Szybalski, E., M. Fiandt, **A. Honigman,** E. Rosenvold and W. Szybalski (1977). A deletion of the p-Q (nin) region of phage lambda b2mm21 conferring partial N21 independence. Gene. 2:294-296.

12. Fuchs, C., E. Rosenvold, **A. Honigman** and W. Szybalski (1979). A simple method for identifying the palidromic sequences recognized by restriction endonucleases: the nucleotide sequence of the Ava 11 site. Gene. 4:1-23.

13. Honigman, A., S.L. Hu and W. Szybalski (1979). Regulation of integration by coliphage lambda. Virology 92:542-556.

14. Enquist, L., **A. Honigman,** S.L. Hu and W. Szybalski (1979). Expression of int gene function in ColE1 hybrid plasmids carrying the C fragment of bacteriophage lambda. Virology 92:557-560.

15. Fuchs, C., E. Rosenvold, **A. Honigman** and W. Szybalski (1980).Identification of palidromic sequences recognized by restrictases, as based on the tabularized sequencing data for seven viral and plasmid DNAs. Gene. 10:357-370.

16. **Honigman, A.,** A. Oppenheim, B. Hohn and T. Hohn (1981). Plasmid vectors for positive selection of hybrid plasmid: Specified cloning vehicle for isolation of transcriptional determination signals. Gene. 13:289-298.

17. **Honigman, A.** Cloning and characterization of an N-resistant transcriptional terminator in bacteriophage lambda. Gene. 13:299-309.

18. Hyman, H. and A. **Honigman** (1982). The use of the plasmid pHA10 in the isolation of lambda PL promoter mutations. Mol. Gen. Genet. 185:515-517.

19. Becker, Y., Y. Shtram, D. Snipper, Y. Asher, E. Tavor, Y. Gordon, D.Gilden, M. Vallish, J. Hadar, O. Becker, A. Cohen, A. Laban and **A. Honigman**(1982). Herpes Simplex virus type 1 Thymidine kinase gene controls expression in eukaryotic and prokaryotic cells and viral pathogenesis in mice In: International Symposium of Herpes Virus: Clinical, Pharmalogical and Basic Aspects. Tokushima City, Japan. Ed. W. Prusoff, Y.C. Cheng, H.Shiota, Experta Medica, Amsterdam, The Netherlands, pp. 57-73.

20. **Honigman, A**. and A. Panet (1983). A unique sequence in murine leukemia virus long terminal repeat functions as a termination signal for transcription in Escherichia coli. J. Virol. 45:456-461.

21. Becker, Y., Y. Shtram, **A. Honigman**, A. Laban and A. Cohen (1983)Expression of herpes simplex tk gene in E. coli. Gene. 21:51-58.

22. **Honigman, A.,** C. Kronman, I. Nur, N. Greenberg and S. Rottem (1984).Cloning of L-2 DNA in Escherichia coli pOL4 plasmid. Isr. J. Med. Sci. 20:793-796.

23. Broza, M., B. Sneh, A. Yawetz, U. Oron and **A. Honigman** (1984).Commercial application of Bacillus thuringiensis var. entomocidus to cotton fields for the control of Spodoptera littoralis Boisduval (Lepidoptera,Noctuidae). J. Econ. Entom. 77:1530-1533.

24. Oron, U., M. Skolover, A. Yawetz, M. Broza, B. Sneh and **A. Honigman** (1985). Ultrastructural changes in larval midgut epithelium of Spodoptera littoralis following ingestion of endotoxin of Bacillus thuringiensis var.entomocidus. J. Invertebr. Pathol. 45:353-355.

25. **Honigman, A.,** A. Bar-Shira, H. Silberberg and A. Panet (1985). Generation of a uniform 3' and RNA of murine leukemia virus. J. Virol.53:330-334.

26. **Honigman**, **A**., J. Mahajna, S. Altuvia, S. Koby, D. Teff, H.Locker-Giladi, H. Hyman, C. Kronman and A. Oppenheim (1985). Plasmid vectors designed for the analysis of transcription termination signals. Gene. 36:131-141.

27. Oppenheim, A.B., J. Mahajna, S. Altuvia, S. Koby, D. Teff, H.Locker-Giladi, H. Hyman, C. Kronman and **A. Honigman** (1985). Utilization of control elements for gene expression studies in E. coli, in: Recombinant DNA Research and Viruses, Y. Becker, Editor. Martinus Nijhoff Publishing,Boston, pp. 73-86.

28. **Honigman, A.**, H. Hyman and A.B. Oppenheim (1985). Analysis of viral transcription-termination signals, in: Viral Messenger RNA, Y. Becker,Editor. Martinus Nijhoff Publishing, Boston, pp. 61-75.

29. **Honigman, A.** and G. Nedjar-Pazerini (1986). Cloning and expression of the Lepidoptecan toxin produced by B. thuringiensis in E. coli. Gene. 42:69-77.

30. Hyman, H. and **A. Honigman** (1986). Transcription termination and processing sites in the lambda PL operon. J. Mol. Biol. 189:131-141.

31. Mador, N., A. Panet and A. **Honigman** (1989). Translation of gag pro and pol gene products of human T-cell leukemia virus type 2. J. Virology 63:2400-2404.

32. Unge, T., H. Ahola, K. Bhikhabhai, S. Lovgren, E. Fenyo, **A. Honigman,**A. Panet, J. Gronowirz and B. Strandberg (1990). Expression, purification and crystallization of the HIV-1 reverse transcriptase (RT). Aids Research and Human Retroviruses 6:1297-1303.

33. **Honigman, A.,** D. Wolf, S. Yaish, H. Falk and A. Panet (1991).cis-acting RNA sequences control the gag-pol translation readthrough in murine leukemia virus. Virol. 183:313-319.

34. Israel, S. and **A. Honigman** (1991). A bioluminescence assay for gene expression by continuous growing mammalian cells: application in detection of human immunodeficiency virus type I. Gene, 104:139-145. 35.

35. Bar-Shira, A., A. Panet and **A. Honigman** (1991). An RNA secondary structure juxtaposes two remote genetic signals for HTLV-I RNA 3' end processing. J. Virol. 65:5165-5173.

36. Falk, H., N. Mador, R. Udi, A. Panet and **A. Honigman** (1993). Two cis-acting signals control ribosomal frameshift between human T-cell leukemia virus type II gag and pro genes. J. Virol. 67: 6273-6277.5.930, 2/28, 19, 18

37. Bentwich Z., Lawatti, A., Vonsover S., **Honigman A.**, Israel S., (1993) Tat detection assay by Luminescence. J. ACQ Immun. Def. Synd and Human Retroviruses. 6: 728

38. Kollmus, H., **A. Honigman,** A. Panet and H. Hauser (1994). Sequence and distance of slippery site and stem-loop define the strength of ribosomal frameshifting in HIV-1 and HTLV-2 in vivo. J. Virol. 68:6087-6091. 5.930, 2/28, 19;18, 5.930, 2/28, 16, 15

39. **Honigman, A.,** H. Falk, N. Mador, T. Rosental and A. Panet (1995). Translation efficiency of the human T-cell leukemia virus (HTLV) gag gene modulates the frequency of ribosomal frameshifting. Virology 208:312-318. 3.507, 5/28, 2, 2

40. Israel, S., M. Mendelovitz and **A. Honigman**(1995). Transactivation of human T-cell leukemia virus type 1 by Herpes simplex virus type 1. Virus Genes 9:269-276. 1.342, 20/28, 81/114, 2, 2

41. Nir-Paz, R., S. Israel, **A. Honigman** and I. Kahane (1995). Mycoplasmas regulate HIV-LTR-dependent gene expression. FEMS-Microbiol. Letters 128:63-68. 1.615, 39/83, 7, 7

42. Wolf, D.G., **Honigman, A.,** Lazarovits, J., Tavor, E. and A. Panet (1998). Characterization of the human cytomegalovirus UL97 gene product as a virion-associated protein kinase. Archives of Virology. 143:1223-1232.1.705, 17/28, 8, 8.

43. Wolf, D.G., Yaniv, I., **Honigman, A.,** Kassis, I., Schonfeld, T., and S.Ashkenazi. (1998). Early emergence of ganciclovir-resistant human cytomegalovirus strains in children with primary combined immunodeficiency. J. Infect. Dis.178: 535-538. 4.988, 2/36, 12, 12

44. **Honigman, A**. Panet A. (1998) Structur and replication of HIV, In Aids, a global phenomenon. Editors: Morag, A and **Honigman,A**, Academon, The Hebrew University of Jerusalem, Student Union press., Jerusalem, Israel.pp.31-46

45. Axelrode J., **Honigman, A.,** (1999) A sensitive and versatile bioluminescence bioassay for HIV-1 infection carried on Adenoviral vectors. AIDS research and human retroviruses.15: 759-767.

46. Goren, I.,Tavor, E., and **Honigman, A.,** (1999) Gene regulation mediated by interaction between HTLV-1 promoter elements and transcription factors TAX and CREB. Virology, 258:303-312

47. Segev, H, **Honigman, A.** Rosen, H. and Leitersdorf, A. (2000) Transcription regulation of sterol 27-hydroxylase gene and promoter mapping. Atheriosclerosis, 156: 339-347.

48. Wolf DG, Yaniv I, Ashkenazi S, **Honigman A**.(2000) Emergence of multiple human cytomegalovirus ganciclovir-resistant mutants with deletions and substitutions within the UL97 gene in a patient with severe combined immunodeficiency. Antimicrobial Agents and Chemotherapy ; 45:593-595.

49. **Honigman A**, Zeira E, Ohana P, Abramovitz R, Tavor E, Bar I, Zilberman Y, Rabinovsky R, Gazit D, Joseph A, Panet A, Shai E, Palmon A, Laster M, Galun E..(2001) Transgene Expression in Live Animals. Mol Ther ;4:239-249.

50. Goren I, Tavor E., Goldblum A. and **Honigman A.,** (2001) Two Cysteine Residues in the DNA-Binding Domain of CREB Control Binding to CRE and CREB Mediated Gene Expression , J. Mol. Biol., 313: 695-709. 5.359; 42/266

51. Shai E., Falk H., **Honigman A.,** Panet A. and Palmon A.,(2002) Gene transfer mediated by different viral vectors following direct cannulation to mouse submandibular salivary glands. European Journal of Oral Sciences, 110:254-260

52. Bar I , Zilberman Y., Zeira E, Galun E., **Honigman A**, Turgeman G., Clemens T. ,Gazit Z., Gazit D., (2003) Molecular imaging of the skeleton: Quantitative real time bioluminescence Monitoring gene expression in bone/skeletal repair and development. J Bone Miner Res 18: 570-578.

53. Wolf D, Lurain NS. Zuckerman T., Hoffman R., Saitinger J., **Honigman A.,** Saleh N., Robert ES., Rowe JM., Kra-Oz Z., (2003) Emergence of late cytomegalovirus central nervous system disease in hematopoietic stem cell transplant recipients.Blood,101:463-465.

54. Evelyne Zeira, Alexandra Manevitch, Artium Khatchatouriants, Orit Pappo, Esti Hyam, Dina Ben-Yehuda, Einat Tavor, **Alik Honigman**, Aaron Lewis, and Eithan Galun.(2003), Femtosecond infrared laser - an efficient and safe in vivo gene delivery system for prolonged expression. Mol Ther.,8:342-349.

55. Matza-Porges S, Tavor E, Panet A and **A Honigman A**.(2003) Intracellular expression of a short peptide inhibits caspase3 activity and confers resistance to apoptosis. Exp.cell res., 290:60-67

56. Aframian DJ, Amit D, Shai E, Baum BJ, Panet A, **Honigman A**, Deutsch D, Palmon A, (2003), Enhancement of functional firefly luciferase secretion from salivary epitheial cells. Journal of dental research 82: 379-379 , 2.702,

57. Abramovitz R., Tavor, E., Jacob-Hirch, J., Zeira, E., Amariglio, N., Pappo, O., Rechavi, G., Galun, E., and **Honigman, A**. (2004) A pivotal role of CREB in Tumor progression.. Cancer Res. 64: 1338-1346,

58. Borkow G., Lara H.H., Ayash-Rashkovsky M., Tavor E., Lapidot A., Bentwich Z. and **Honigman A.** (2004). Adenovirus bioluminescence reporter gene and cMAGI cell assay for the detection of HIV-1. Virus Genes 29: 257-265,

59. Amos Panet; Efrat Braun; **Alik Honigman,** and Israel Steiner (2005). Involvement of Cellular Death Signals in the Reactivation of Herpes Simplex Virus Type 1 and Lambda Bacteriophage from a Latent State. Journal of Theoretical Biology 236: 88-94,

60. Tal Arnon, Hagit Achdout, Ofer Levi, Gal Markel, Niveen Saleh, Gil Katz, Roi Gazit, Tsufit Gonen-Gross, Jacob Hanna, Efrat Nahari, Angel Porgador, **Alik Honigman**, Bodo Plachter, Dror Mevorach, Dana G Wolf and Ofer Mandelboim. (2005). Interaction between the HCMV pp65 protein and the NK activating receptor NKp30 leads to general inhibition of NK cytotoxicity Nat Immunol. 6: 515-523.

61. Sigal Matza-Porges, Ido Horresh, Einat Tavor, Amos Panet and **Alik Honigman**. (2005). Expression of an Anti Apoptotic Recombinant Short Peptide in Mammalian Cells. Apoptosis, 10:987-996.

62. Maysa Azzeh, **Alik Honigman**, Albert Taraboulos, Alexander Rouvinski and Dana G. Wolf . (2006) Human Cytomegalovirus UL97 Kinase is involved in Remodeling of Viral Cytoplasmic Assembly Sites. Virology, 354:69-79.

63. Soreq H., **Honigman A**., (2007) It is all about resolution. FEBS J.; 274:924-927.

64. D.J. Aframian, D. Amit, R. David, E. Shai, D. Deutsch, A. **Honigman, A**. Panet, and A. Palmon, (2007) Re-Engineering Salivary Gland Cells to Enhance Protein Secretion For Use in Developing Artificial Salivary Gland Device. Tissue Engineering. , 13, Issue 4 (April 2007).